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Some biochemcial studies on the human lens nucleus

Xiaojia Wei
University of Wollongong

SOME BIOCHEMCIAL STUDIES ON THE HUMAN LENS NUCLEUS

Wei Xiaojia (Eric), B.Sc., M.Sc.

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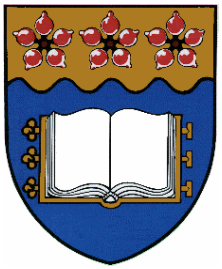

Supervisor: Professor Roger Truscott

Department of Chemistry

University of Wollongong

Wollongong, Australia

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CERTIFICATION

I, Xiaojia Wei, declare that this thesis, submitted in full fulfillment of the requirements for the award of Master of Science by Research, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

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LIST OF ABBREVIATIONS

The following abbreviations were used in this thesis

ACRF	Australian Cataract Research Foundation
ADP	Adenosine Diphosphate
Ag/AgCl	Silver/Silver Chloride
AHBG	4-(2-amino-3-hydroxyphenyl-4-oxobutanoic acid glucoside
AQP0	Aquaporin0
ARNC	Age-related Nuclear cataract
ATP	Adenosine Triphosphate
BCA	Bicinchoninic Acid
BSA	Bovine Serum Albumin
$CHCl_3$	Chloroform
CoQ	CoenzymeQ
CytC	CytochromeC
DC Voltage	Direct Current Voltage
DI	Direct insertion
DNP	2,4-dinitrophenol
DTNB	5,5'-dithiobis-(nitrobenzoic acid)
EDTA	Ethylenediaminetetraacetic Acid
EI Ionization	Electron Impact Ionization
ESI	Electrospray Ionization
ETC	Electron Transport Chain
FCCP	Carbonylcyanide-p-trifluoromethoxyphenyl hydrozone
GSH	Glutathione (reduced form)
GSSG	Glutathione (oxidized form)
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HMM	High Molecular Mass
HPLC	High Performance Liquid Chromatography
IAA	Iodoacetic Acid
IOL	Intraocular Lens
KCl	Potassium Chloride
Kyn	Kynurenine
MALDI	Matrix Assisted Laser Desorption Ionization
MeOH	Methanol
m/z	Mass to Charge
NADH	Nicotinamide Adenine Dinucleotide (reduce form)

NMR	Nuclear Magnetic Resonance
O_2	Molecular Oxygen/Oxygen
PBS	Phosphate-buffered Saline
pO_2	Partial Pressure of Oxygen
PSH	Peotein Sulphydryl
R^2	Regression
Rf Voltage	Radio Frequency Oscillation Voltage
ROS	Reactive Oxygen Species
SDS	Sodium Dodecyl Sulphate
SIM	Selected Ion Monitoring
TCA	Trichloracetic Acid
Tris-HCl	Tris(hydroxymethyl)aminomethane
UV	Ultra Violet
WR	Working Reagent
WS	Water Soluble
y.o.	Years Old

ABSTRACT

Barrier[16] formation has been shown to occur in the lens with age. It is important to understand the physiological changes in the lens that occur upon the formation of the barrier and their implication on the onset of cataract and presbyopia (old man's eyes). In this study, three factors related to the formation of the barrier were investigated: diffusion rate changes in the lens nucleus, oxygen consumption and cholesterol compositional changes in the lens with age.

A Franz Cell was used to measure the diffusion rate changes in the nucleus of the human lens. No significant differences in the rate of diffusion between young and old lenses could be detected with this technique.

The role of protein sulphydryls as secondary oxygen consumers was also studied. It was shown that protein sulphydryls reacted readily with oxygen, suggesting that protein sulphydryls are a secondary O_2-consumption system in the center of the lens. Mitochondria are the primary oxygen consumers in the lens.

A technique for the quantification of cholesterol in lipid extracts was developed. Results obtained were comparable to published results using traditional methods. The concentration of cholesterol in the young human lens was found to be approximately 3-fold greater than that of the bovine, ovine and porcine lenses, and ~5 times greater

than in the gallinaceous lens. These differences were even more pronounced when an elderly human lens was examined. The nucleus of the human lens was found to have a higher level of cholesterol content than that in the cortex and the concentration of cholesterol also exhibited a significant increase with age in both nuclear and barrier regions.